Determination of Solvents in Pharmaceuticals by Static Headspace GC

GC

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In late 1990, the United States Pharmacopeia released two methods[1] for determining organic volatile impurities in pharmaceutical compounds by gas chromatography. The first method involved a liquid-liquid extraction prior to the analysis and the second utilized the purge and trap or dynamic headspace technique. The solvents specified in the methods and the maximum allowed quantities are shown in Table 1. This application note demonstrates that static headspace (SHS) offers an alternative approach to this determination.

Instrumentation and Conditions

Instrument: Varian Genesis Headspace AutoSampler connected to a Varian 3400 gas chromatograph. A model 8100 AutoSampler (for liquid sample introduction) was mounted on the GC. A GC Star Workstation with the Advanced Applications Package (Excel® Macros) was used for data handling.

Headspace Sampler: Samples 60°C, line and valve 150°C, equilibration time 4 min, mixing at 80% of full power for 7 min, post mixing stabilization time 1 min. Sample vials were 22 mL with 8 mL of sample. Sample loop was 500 µL.

Injector: Septum-equipped temperature-programmable injector (SPI) with high performance capillary insert (160°C). The transfer line of the SHS system was connected directly to the carrier gas inlet of the SPI. This allowed control of the column flow by the Genesis flow controller and left the injector nut accessible for manual or liquid autosampler injections.

Column: 30m x 0.32 mm coated with 0.5 µm PEG Varian Equivalent: CP-Wax 52 CB, #CP8763), Column outlet connected with a QuickSeal Y Splitter to an FID and ECD. Oven: 35°C(0 min), 5°/min to 60°C

Carrier gas: Helium at 71 cm/sec.

Detectors: 180°C, FID at ranges 10^{-12} and 10^{-11} and ECD at ranges 1 and 10, depending on sample concentration.

Samples

Test samples: To determine optimum headspace parameters, a test sample was prepared. The solvents in Table 1 were added to HPLC grade water. The test sample was stored in a 1.2-L bottle that was filled to the top. To determine the quantities of each organic compound actually dissolved in the test sample, a comparison was made with another standard. This "absolute" standard was prepared by adding 20 µL of each solvent to CS_2 and diluting to 25 mL. The solvents were all known to be soluble at these levels in CS_2. FID response factors were generated by injection of the liquid into the GC. Next, the aqueous standard was injected directly into the GC. The response of each solvent was compared to that of the same solvent in the CS_2 and the concentration was calculated.

Pharmaceuticals: Two different procedures were used to prepare pharmaceutical samples and the results were compared.

1. **Sampling over a liquid.** One gram of dry compound (an antacid, a cephalosporin antibiotic and tetracycline) was mixed with 5 mL HPLC water to form a slurry. Organic solvent impurities were determined by comparing headspace over the slurry with the headspace over the aqueous standard.
2. **Sampling over the solid pharmaceutical.** The dry samples were spiked with 1 µL of n-butanol, containing the USP solvents to obtain final concentrations of 10 ppm (chloroform 5 ppm), and 100 ppm (chloroform 50 ppm). The samples were also spiked with a 1-µL n-butanol blank. The headspace over these mixtures was sampled utilizing the parameters described above.

Procedure

Optimization studies: The method optimization[2] feature of the Genesis was utilized to establish the headspace parameters.

Linearity and minimum detectable quantity (MDQ): The linearity study was undertaken with the aqueous test sample and seven dilutions down to approximately 1 ppb.

All of the glassware and water used for the dilutions were refrigerated to minimize losses of volatiles during the sample handling.

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Results and Discussion

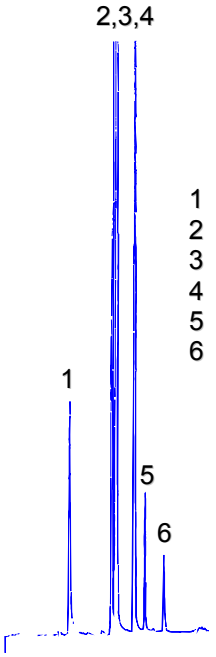
Figure 1 is a chromatogram of the headspace over the aqueous test sample.

FID linearity was demonstrated with all of the solvents with correlation coefficients to a straight line greater than 0.999 in all cases. The Excel macro with multi-level calibration was used to generate a calibration curve for the three compounds eliciting a response with the ECD.

The area count precision and MDQ's are shown in Table 1. The area count precision was excellent especially when one considers the potential loss of volatile solvents due to sample handling. The MDQ's for all of the solvents easily met the criteria in the USP method. MDQ results for most of the solvents were similar when the compounds were spiked into the solid pharmaceuticals. However, ethylene oxide was not detected. At this time, the reason for this is unknown. Possibly, there was breakdown at the temperature (80°C) of the dry sample.

Dioxane is quite soluble in water and could be detected in ten times the quantity shown if spiked into the dry sample. The MDQ of dioxane in water could be enhanced by saturating the aqueous solution with Na_2SO_4, by raising the sample temperature from 60° to 80°C or by using a smaller volume of water.

In situations where trichloroethylene and chloroform must be detected at low ppb levels, the ECD would prove useful.



		RT	ppm/Water
1	Ethylene Oxide	1.59	76
2	Methylene Chloride	2.64	110
3	Benzene	2.74	47
4	Trichloroethylene	3.21	79
5	Chloroform	3.48	47
6	Dioxane	3.96	118

Figure 1. Static Headspace Chromatogram of USP Solvents

Conclusion

In conclusion, SHS is a simple technique for detecting organic volatile impurities in pharmaceuticals and easily meets the sensitivity requirements of the USP.

References

1. The United States Pharmacopeia, 22nd revision, Supplement 3, Rockville, MD, 20852 (1990) p. 2395.
2. Optimization of Parameters in Static Headspace GC, GC Application Note #40, Zelda Penton.

Table 1. Precision and MDQ's (s/n=5) of Each Organic Impurity are Given for 1-gram Samples of Pharmaceutical Compound in 5 mL of Water

Compound	USP Limit (ppm)	ppm in Test Sample	RT (min)	%RSD n=5 (Conc. ppm)	MDQ (ppb) FID	MDQ (ppb) ECD
Ethylene oxide[a]	10	76	1.45	2.89 (76)	$3x10^3$	
Methylene Chloride	100	74	2.21	1.22 (15)	38	8.5
Benzene	100	40	2.31	1.16 (0.8)	3	
Trichloroethylene	100	21	2.91	1.25 (0.4)	16	0.02
Chloroform	50	51	3.30	1.32 (1.0)	47	0.03
Toluene[b]	—	41	3.55	1.22 (0.8)	3	
Dioxane	100	122	3.92	4.25 (2.4)	$5x10^3$	

[a] Ethylene oxide was purchased as the pure material in a lecture bottle. The lecture bottle was refrigerated so that the gas condensed to a liquid. To prepare standards liquid ethylene oxide was removed with a chilled syringe and injected into the solvent. Ethylene oxide was measured only in a preliminary study with a 50-µL sample loop. The MDQ should be enhanced by a factor of 10 with the 500-µL loop.

[b] Toluene was added to the mixture since it has replaced benzene as a solvent in most laboratories.

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These data represent typical results.
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